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SOME ASPECTS OF POSITION PSEUDOALLELISM*

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INTRODUCTION

The phenomenon of pseudoallelism promises to contribute much to our understanding of the gene—how it functions, how it mutates, how it evolves. The functional aspect will be the central theme of this paper. Attention will be focused chiefly on examples of "position pseudoallelism;" namely, those cases in which there is a position effect or phenotypic difference between the "cis-type" ($ab/++$) and "trans-type" ($a+/+b$) of double mutant heterozygote. In most of these examples a close functional relationship between the adjoining loci is indicated.

There are currently two contrasting interpretations of position pseudoallelism. On the first or functional interpretation the mutants at the different pseudoallelic loci are alterations at different sites of a single functional unit which is called the gene. On the second or genetic interpretation the mutants at the different loci are alterations in different units each of which is called a gene, whether it be a single functional unit or not.

The functional interpretation is currently advocated by Pontecorvo (1952) on the basis of studies (Roper, 1950; and Roper in Pontecorvo et al., 1953) of three biotin mutants in *Aspergillus nidulans*, which are presumptive position pseudoalleles although recovery of cis-types has not been reported; and by MacKendrick and Pontecorvo (1952) on the basis of studies of certain pairs of white "alleles," which, by analogy with the case of apricot and white (Lewis, 1952), may be assumed to be position pseudoalleles. The chief difficulty with this type of interpretation is that adequate criteria for recognizing a functional unit are not available. Mere appearance of functional identity, as in the case of the above biotin mutants, or certain inositol-less mutants in Neurospora which are also presumptive position pseudoalleles (Giles, 1951), is obviously not sufficient. Thus, it is easy to see how two or more units which control different reactions in a sequential series can mimic the action of a single unit. Another criterion has been the phenotypic test of allelism; thus, two recessive mutants, each arising independently from a standard or wild type, have been considered alleles of a single gene if the heterozygote between them has a mutant phenotype. This criterion is inadequate since it does not take into account the possibility of position effects; thus, the finding that the trans-type in the case of position

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pseudoallelism is mutant in phenotype is readily explained by a position effect involving the products of two different functional units (Lewis, 1951).

Paradoxically, the application of the phenotypic test of allelism has largely been responsible for endowing the gene with complex functional attributes rather than a unity of function. Thus, most of the well-studied cases of multiple allelism (or pseudoallelism, as the case may be) that have been identified largely by means of this test show evidence for at least two, more or less independently varying, functional components; for example, the dumpy mutants (Muller, 1922) and the scute and achaete series of mutants (beginning with the work of Serebrovsky, 1930) in Drosophila, and the "*R*" mutants (Emerson, 1921; Stadler, 1946; 1953; 1954) and the "*A*" mutants (see Laughnan in this Symposium) in maize. The difficulties in setting up criteria for functional unity become more aggravated in the case of allelic or pseudoallelic series of dominant mutants and/or mutants of obscure origin. Such cases tend to show even greater degrees of complexity at the functional level; *e.g.*, self-sterility alleles in Oenothera (D. Lewis, 1949; and others), the "E" mutants in Bombyx (reviewed by Tanaka, 1953) and the genes controlling cellular antigens in cattle (Stormont, Owen and Irwin, 1951).

The evidence from established cases of position pseudoallelism in Drosophila suggests not a functional unity but as many functional components as there are different loci. This has been discussed in detail before (Lewis, 1951) for the cases of Star-asteroid (Lewis, 1945), Stubble-stubbloid, three loci of the bithorax series, and the three loci of the lozenge series investigated by Green and Green (1949). In the lozenge case the evidence is incomplete but already points to at least two functional components (fertility and eye effects). The more recent example of apricot-white pseudoallelism, referred to above, well illustrates the same point, since the series of white "alleles" has long been known (Morgan, et al., 1931) to be separable into two qualitatively distinct groups with respect to reaction to Bridges' specific modifier gene, Pale, or to sexual dimorphism. Thus, the eye-color of mutants of the "apricot" group (including apricot, blood, coral and honey) is darker in the male than the female, while it is lighter in the male than in the female in the case of the "eosin" group (including white, as well as eosin and most of the other mutants of the series). The data of MacKendrick and Pontecorvo (1952) can be interpreted to mean that coral and blood each lie to the left of white, parallelling the finding that apricot lies to the left of white. To this limited extent, at least, the separation of the white series by the crossing-over test coincides with the separation by the test of qualitatively different function. In the recent case of two vermilion position pseudoalleles, Green (1954) has used a more or less specific modifier gene to distinguish the two; but the evidence for functional differentiation is not quite so convincing in this case. Finally, there is the probable case of two singed position pseudoalleles discovered by Ives and Noyes (1951), which awaits report of the cis-type; here, too, the evidence from existing mutants

(see Bridges and Brehme, 1945) suggests two, more or less independently varying attributes (fertility and bristle effects).

By contrast the genetic interpretation refers the complexity of functioning which typifies pseudoallelism to interactions at the level of different gene products. The standard operational criteria for defining the gene—indivisibility by the crossing-over or rearrangement test—are thus still preserved; and there remains a one-to-one correspondence between gene and locus.

Specifically, a model of gene action in terms of sequential biochemical reactions has proved a fruitful working hypothesis. This kind of model was formerly advocated by Pontecorvo (1950) on the basis of theoretical considerations of millimicromolar reactions (McIlwain, 1946), and was independently put forward (Lewis, 1949) and elaborated in some detail (Lewis, 1950; 1951) to explain the position effect which characterizes position pseudoallelism. This model assumes that (1) the normal allele of one of the pseudoallelic genes, a^+, controls a reaction: S $\longrightarrow$ A, while the normal allele of a second gene of the series, b^+, controls a reaction: A $\longrightarrow$ B; (2) the mutant alleles block or impair these reactions; and (3) the substance A, at least, is produced at, or very close to, the site of the gene in the chromosome and is effectively transported along the chromosome more readily than it is transported to the homologous chromosome. As may be seen from the diagrams below, the cis-arrangement of the wild-type alleles (1) is expected on the above assumptions to give a more nearly normal action (production of substance B) than the trans-arrangement (2):

(1)

$$\begin{array}{l} S \xrightarrow[]{a^+} A \xrightarrow[]{b^+} B \\ \hline S \xrightarrow[a]{} {<}A \xrightarrow[b]{} {<}B \end{array}$$

(2)

$$\begin{array}{l} S \xrightarrow[]{a^+} A \xrightarrow[]{b} {<}B \\ \hline S \xrightarrow[a]{} {<}A \xrightarrow[b^+]{} {<}B \end{array}$$

A new kind of supporting evidence for this model has come from the discovery that, in the case of the bithorax pseudoallelic series, structural heterozygosity for certain chromosomal rearrangements (R) significantly alters the phenotype of particular trans-types towards a more extreme departure from wild-type, yet, in general, does not alter the phenotype of the cis-type. This new type of position effect, detected by comparing $R(a+)/+\,b$ or $a+/R(+\,b)$, on the one hand, with $a+/+\,b$ on the other, will be referred to as the "trans-vection effect." As recently described in some detail (Lewis, 1954b), the majority of X-ray-induced rearrangements which have at least one breakage point between the centromere and the locus of *bx* (a distance of over 500 "bands" of the salivary gland chromosomes) evoke the trans-vection effect; moreover, the results of the analysis of these strongly supports the hypothesis that a reduction in somatic pairing is the causative factor in modifying the phenotype of the trans-type. The trans-vection effect phenomenon is readily understandable on the above model by assuming

that a reduction in somatic pairing effectively blocks the residual transport of substance A (diagram 2, above) from its site of production in one chromosome to the corresponding site in the homologous chromosome.

The position effect which is detected by comparing the cis- and trans-type and which is the basis for defining position pseudoallelism now needs a term to distinguish it from the trans-vection effect, and will henceforth be referred to as the "cis-vection effect" (Lewis, 1954b). A variety of cis-vection and trans-vection effects are known in the bithorax case, and the remainder of this paper will be devoted to a systematic treatment of these phenomena.

CIS-VECTION EFFECTS

Evidence for five loci in the bithorax pseudoallelic series has recently been presented (Lewis, 1954a). In order from left to right starting at locus 58.8 in the third chromosome, the loci are: bithorax (*bx*), Contrabithorax (*Cbx*), Ultrabithorax (*Ubx*), bithoraxoid (*bxd*) and postbithorax (*pbx*). The evidence was, however, incomplete in that between two pairs of genes, *Cbx* and *Ubx*, and *bxd* and *pbx*, only the wild-type crossovers had been recovered from females of the trans-type. Since then the complementary crossovers, *Cbx Ubx* and *bxd pbx*, respectively, have been obtained from females of the appropriate trans-type. Cis-vection effects have been studied by comparing all ten possible double mutant combinations in the cis- and trans-types for the mutants, bx^3, *Cbx*, *Ubx*, *bxd* and *pbx*. Before considering them, however, the individual mutant phenotypes will be briefly reviewed.

Three of the mutants, bx^3, *Ubx* and *bxd*, are spontaneous in origin and have been described in some detail before (see Bridges and Brehme, 1944; Lewis, 1951; *Ubx* was formerly designated as bx^D, in the former reference; as *Bxl*, in the latter; and as bxd^D by Lewis, 1949). The *Cbx* and *pbx* mutants are of X-ray origin, and, as recently reported (Lewis, 1954a) are remarkable in that they appear to have been induced simultaneously. Each of these five mutant genes appears to be normal in the salivary gland chromosomes. These genes effect well-defined transformations of certain body segments or parts of body segments. At least four sharply distinct and more or less independently varying transformations of this kind have been recognized. On the basis of these, each mutant can be rather precisely described and readily distinguished from any one of the others (see table 1). Thus, the bx^3 homozygote has the anterior portion of the metathorax (AMT) transformed into a structure which very closely resembles the anterior portion of the mesothorax (AMS)—a transformation (Type I) which will be symbolized: AMT $\rightarrow$ AMS. The *pbx* homozygote has a second type of transformation (Type II); namely, a conversion of the posterior portion of the metathorax (PMT) into a structure very closely resembling the posterior portion of the mesothorax (PMS), or symbolically: PMT $\rightarrow$ PMS. The *bxd* homozygote also has this Type II transformation, but not quite so well developed, and, in addition, always has a thoracic-like modification of the

TABLE 1

INDIVIDUAL MUTANT PHENOTYPES OF THE BITHORAX PSEUDOALLELIC SERIES

Legend: 0 = little or no transformation; hence wild-type, or nearly so; +, ++, +++, ++++ = very slight, slight, moderate, extreme degrees, respectively, of the indicated transformation.

Name of locus	Genotype	Type of body segment transformation			
		I	II	III	IV
bithorax	bx^3/bx^3	++++	0	0	0
postbithorax	pbx/pbx	0	++++	0	0
bithoraxoid	bxd/bxd	0	+++	+++	0
Contrabithorax	Cbx/+ and Cbx/Cbx	0	0	0	++++
Ultrabithorax	Ubx/+	+	0	0	0

first abdominal segment (AB_1). The latter transformation (Type III) is primarily towards a structure resembling AMT, and will be symbolized: $AB_1 \rightarrow$ AMT; however, the presence of posteriorly wing-like halteres on AB_1 (thus far found only in bxd/bxd^{121}, where bxd^{121} is of X-ray origin) implies that posteriorly AB_1 changes towards PMS. The *Ubx*/+ genotype has an extremely slight Type-I transformation (recognizable only in the haltere, whose distal segment is enlarged and more hairy on the anterior margin than the wild-type haltere). The *Ubx* homozygote is lethal in the adult stage but the larval phenotype and interactions with other mutants of the series indicate that it is phenotypically like double-mutant homozygotes between *bxd* and a *bx* mutant; *i.e.*, combines Type-I, -II, and -III transformations (the combination of Type I and Type III giving a transformation of AB_1 towards AMS). Whether *Ubx* differs qualitatively from such double mutant combinations is not clear. The *Cbx* homozygote, as well as the virtually identical *Cbx*/+ genotype, has a fourth transformation (Type IV); namely, a reduction in the development of PMS so that it partially resembles, especially in the case of the wing, PMT—or a change in the mesothorax which may be written: PMS $\rightarrow$ PMT. Occasionally, the *Cbx* phenotype has also a reduction in AMS so that the latter begins to resemble AMT, especially in the case of the wing which becomes almost completely haltere-like.

Since the first known mutant of the above series, bx^1 of Bridges (see Bridges and Brehme, 1944), is highly variable and may occasionally overlap wild-type, the false impression may have arisen in some quarters that these so-called homeotic mutants are intrinsically highly variable. On the contrary, all of the above described mutant effects are surprisingly uniformly expressed, with the exception of the variability noted for the *Cbx* mutant. In no case have any of these phenotypic effects, including those of the *Cbx* mutant and the slight dominant effect of *Ubx*/+, been observed to overlap the wild-type phenotype.

With the above five mutant genes and their ten possible double mutant combinations, there are ten possible pairs of cis- and trans-types to be compared for cis-vection effects. All ten pairs have been constructed and their phenotypes are summarized in table 2 in terms of four transformation types

TABLE 2

CIS-VECTION AND TRANS-VECTION EFFECTS INVOLVING THE BITHORAX PSEUDOALLELIC SERIES (LEGEND AS IN TABLE 1)

Group	Mutants in heterozygote	Type of heterozygote	Type of body segment transformation			
			I	II	III	IV
1.	a. bx^3 and bxd	cis	0	0	0	0
		trans	0	0	0	0
		R-trans	0	0	0	0
	b. bx^3 and pbx	cis	0	0	0	0
		trans	0	0	0	0
		R(cis)	0	0	0	0
		R(trans)	0	0 to +	0	0
	c. bxd and pbx	cis	0	0	0	0
		trans	0	+++	0	0
		R(trans)	0	+++	0	0
2.	a. bx^3 and Ubx	cis	+	0	0	0
		trans	+++	+	0	0
		R(trans)	++++	+	0	0
	b. Ubx and bxd	cis	+	0	0	0
		trans	+	+++	+++	0
		R(trans)	+	+++	+++	0
	c. Ubx and pbx	cis	+	0	0	0
		trans	+	++++	0	0
		R(trans)	+	++++	0	0
3.	a. bx^3 and Cbx	cis	0	0	0	++
		trans	0 to +	0	0	++++
		R(trans)	0 to +	0	0	++++
	b. Cbx and bxd	cis	0	0	0	++++
		trans	0	0	0	++++
		R(trans)	0	0	0	++++
	c. Cbx and pbx	cis	0	0	0	++++
		trans	0	0 to +	0	++++
		R(trans)	0	0 to +	0	++++
4.	a. Cbx and Ubx	cis	+	0	0	+
		trans	++	+	0	+++
		R(cis)	+	0	0	0
		R(trans)	++	+	0	+++

described above. For the sake of systematic presentation, the results will be discussed in terms of the four groups of comparison that can be made on the basis of dominant and recessive relationships.

Group-1 comparisons involve only the recessive mutants. The cis- and trans-types for bx^3 and *bxd* are both wild-type in phenotype; thus, no cis-vection effect is in evidence. The cis- and trans-types for bx^3 and *pbx* are also wild-type; in contrast with the previous comparison, however, these genotypes can be shown to differ phenotypically by making each a structural heterozygote for chromosomal rearrangements which evoke moderate to ex-

treme trans-vection effects. As described elsewhere (Lewis, 1954b), the translocation, T(2; 3) bw^{VDe3}, which has the major portion of the right arm of the third chromosome reciprocally translocated to the distal portion of the right arm of the second chromosome, is such a rearrangement, and is useful to employ since it has an inseparable, dominant, variegated-brown effect. By the use of this rearrangement, it is found that the cis-type, $R(++)/bx^3\,pbx$ remains wild-type; while each of the trans-types, $R(bx^3+)/+\,pbx$ and $bx^3+/R(+\,pbx)$, occasionally has a very slight wing-like development of the posterior region of the haltere; that is, a slight Type-II transformation. Finally, the cis-type, $bxd\,pbx/++$, is wild-type; while the trans-type, $bxd+/+\,pbx$, has a moderate Type-II transformation, thus indicating a strong cis-vection effect.

Group-2 comparisons involve each of the three recessive mutants with the dominant *Ubx* mutant. Each of the three comparisons of this kind shows pronounced cis-vection effects. On the one hand, the three cis-types, $bx^3\,Ubx/++$, $Ubx\,bxd/++$, and $Ubx\,pbx/++$ are phenotypically indistinguishable from each other and from the single dominant mutant heterozygote, *Ubx*/+, which, as already noted, differs from wild-type only by a very slight Type-I transformation. On the other hand, $bx^3+/+\,Ubx$ combines a moderate Type-I (figured by Lewis, 1951) with a very slight Type-II transformation; $Ubx+/+\,bxd$ combines the above very slight Type-I with moderate Type-II and moderate Type-III transformations (since no haltere-like structure has been observed on AB_1 of this genotype, it is not possible to observe whether the very slight transformation of Type I combines in that segment with the Type III one to produce a mesothoracic-like modification of this segment): while the remaining trans-type, $Ubx+/+\,pbx$ combines the very slight Type-I with a very extreme Type-II transformation.

Group-3 comparisons involve each of the three recessive mutants with the dominant *Cbx* mutant. The comparison of $bx^3\,Cbx/++$ with $bx^3+/+\,Cbx$ reveals another type of cis-vection effect. Thus, the trans-type is like the single mutant heterozygote, *Cbx*/+; it has a well-developed Type-IV transformation. The cis-type, on the other hand, has only a slight transformation of this type. The trans-type sometimes also has a very slight Type-I transformation which the cis-type lacks. The trans-types, $Cbx+/+\,bxd$ and $Cbx+/+\,pbx$, show no striking differences from *Cbx*/+, nor from their respective cis-types; however, $Cbx+/+\,pbx$ appears to have the beginning of a Type-II transformation in the region of the haltere, while its cis-type is wild-type in this respect. At the same time, there are striking differences between the cis- and trans-types in each of these two latter comparisons when they are studied in the presence of a recessive, sex-linked, partial suppressor of *Cbx* (symbol, *su-Cbx*; *locus*, $30^{\pm}$; spontaneous in a stock of $v\,Bx^r$; bxd^{51j}, kindly supplied to the author by M. M. Green). Thus, males of the cis-types, *su-Cbx*; $Cbx\,bxd/++$, and *su-Cbx*; $Cbx\,pbx/++$ (as well as the genotypes, *su-Cbx*; *Cbx*/+ and *su-Cbx*; $bx^3\,Cbx/++$) differ from wild-type only in having a very slight Type-IV transformation; that is, they have an

almost complete suppression of the dominant effect of *Cbx*. By contrast, in addition to this latter very slight transformation of Type IV, males of the trans-type, *su-Cbx*; *Cbx+/+bxd*, have a moderate Type-III, and males of the trans-type, *su-Cbx*; *Cbx+/+pbx*, have a slight Type-II transformation. Furthermore, males of the trans-type, *su-Cbx*; $bx^3+/Cbx+$, have a slight Type-I transformation as well as the very slight Type-IV one.

Finally, group-4 comparisons involve the two dominant mutants. The cis-type, *Cbx Ubx/++*, is remarkable in that there is scarcely any detectable Type-IV transformation; the alula of the wing is reduced and the wings are often slightly spread, but the phenotype otherwise is wild-type except for the typical very slight Type-I transformation characteristic of *Ubx/+*. On the other hand, the trans-type, *Cbx+/+Ubx* has a moderate transformation of Type IV (not quite so extreme as that of *Cbx/+*), a slight one of Type I (the haltere being larger and more hairy than that of *Ubx/+*), and a very slight one of Type II.

It is a general rule that cis-vection effects are very striking position effects, in that the cis- and trans-types not only differ strikingly in phenotype, but they show little or no tendency to overlap one another in phenotype over a wide range of environmental conditions. This rule applies to all of the above cis-vections shown in table 2, except that the phenotype of $R(bx^3+)/+pbx$ may overlap wild-type and hence overlap that of $R(++)/bx^3\,pbx$; however if a more complex rearrangement (with respect to structural heterozygosity of the *bx* region) than bw^{VDe3} is employed then there is virtually no overlapping of these two phenotypes.

To summarize, a broad spectrum of cis-vection effects have been met with in the case of the *bx* pseudoallelic series. Both cis- and trans-types for a given pair may prove to be wild-type under perhaps all conditions (bx^3 and *bxd*). This case is analogous to that of miniature (*m*) and dusky (*dy*) mutants studied by Slatis and Willermet (1953) who find that *m+/+dy* and *m dy/++* are each virtually wild-type, although the latter may possibly have significantly shorter wings than the latter. The cis- and trans-types may be wild-type under normal conditions but differ phenotypically when both are made into identical structural heterozygotes (bx^3 and *pbx*). It becomes evident from this case that there may be no sharp line between pseudoallelism with and without the position effect phenomenon: thus, the possibility of position effect is obviously to be kept in mind in the numerous cases of pseudoallelism where both cis- and trans-types are wild-type or otherwise identical in phenotype; e.g., cases of pseudoallelism in mice (Dunn and Caspari, 1942; 1945) or cotton (see reviews of this and other cases by Stephens, 1951; and Komai, 1950). A striking cis-vection effect occurs between two recessive mutants (*bxd* and *pbx*). The latter case is analogous to the cases of lozenge, apricot-white, and vermilion pseudoalleles, already referred to. Striking differences occur in every comparison involving any one of the recessive mutants with *Ubx*; these effects are analogous to those observed with the Star-asteroid series. In comparisons involving *Cbx* unusual

relations arise. Thus, the trans-type may be mutant but not strikingly different from *Cbx*/+; yet position pseudoallelism is indicated by the strikingly different and much more nearly normal cis-type (*Cbx* and bx^3). Or both cis- and trans-types may be mutant and nearly, if not quite, identical; yet cis-vection effects can be revealed in the presence of a sensitizing modifier gene; *su-Cbx*, (*Cbx* and *bxd*, or *Cbx* and *pbx*). *Ubx* acts as a virtually complete suppressor of the dominant effect of *Cbx* in the case of the cis-type, but has only feeble interactions with *Cbx* in the case of the trans-type. This comparison and that involving *Cbx* and bx^3 parallel somewhat the Stubble-stubbloid case (Lewis, 1951); thus stubbloid acts as a complete suppressor of the dominant Stubble phenotype in the case of the cis-type but gives an extreme mutant phenotype in the case of the trans-type.

TRANS-VECTION EFFECTS

To study trans-vection effects, the translocation, bw^{VDe3}, has been used to produce structural heterozygosity. This rearrangement has been combined by crossing over with all of the single mutant types except *Cbx* and most of the double mutant types (the exceptions being *Cbx Ubx*, *Cbx bxd* and *Cbx pbx*). In no case has heterozygosity for this translocation modified the phenotype of a heterozygote for a single mutant gene, and in only one case (discussed below) is the phenotype of the cis-type between two mutants modified. The manner in which certain of the trans-types are altered by heterozygosity for this translocation will be discussed by systematically considering the four groups of double mutant types already adopted. The results are also shown in table 2. In the case of Groups 1 and 2, comparisons have been made in every case between the trans-type without structural heterozygosity, on the one hand, and the two forms of the structurally heterozygous trans-type, or "*R*(trans)-type" as it is designated in table 2, on the other hand. In no case has there been any obvious difference between the two forms of an *R*(trans)-type; that is, between *R*(a+)/+b and *a*+/*R*(+*b*). Both forms of the "R(cis)-type," as the structurally heterozygous cis-type is designated, have also been constructed in all of the Group-1 and -2 cases. Again, in no case involving Groups 1 and 2 has there been an obvious phenotypic difference between such pairs; moreover, since in such cases the *R*(cis)-type does not differ from the cis-type, the R(cis)-type has been omitted from table 2 except where explicitly needed. In the case of Groups 3 and 4 of table 2, only one form of the *R*(trans)-type or *R*(cis)-type has been constructed; and only where the phenotype of the *R*(cis)-type was different from that of the cis-type is it included in the table.

Among the Group-1 comparisons of trans-types with and without structural heterozygosity, only the one involving bx^3 and *pbx* shows a trans-vection effect. In this case, *R*(bx^3+)/+*pbx* occasionally has a very slight Type-II transformation in contrast to the wild-type phenotype of bx^3+/+*pbx*.

Among the group-2 comparisons, the only trans-vection effect yet detected involves bx^3 and *Ubx*. In this case, *R*(bx^3+)/+*Ubx* has an extreme Type-I transformation compared to a moderate Type I found in bx^3+/+*Ubx*.

Among the group-3 comparisons, no obvious trans-vection effects are present. However, preliminary studies utilizing the above-described modifier gene, su-Cbx, have shown that *su-Cbx*; $R(bx^3+)/+Cbx$ males have a significantly more extreme Type-I transformation than su-Cbx; $bx^3+/+Cbx$ males.

Among the Group-4 comparisons, there is one trans-vection effect and it is of a new type. Whereas, in all other examples of this phenomenon, it is only the trans-type that is modified by the appropriate kind of structural heterozygosity, in this case it is only the cis-type which is modified. Thus, the comparison of the trans-type and the R(trans)-type for *Cbx* and *Ubx* reveals no obvious phenotypic difference. On the other hand, the *R*(cis)-type is more nearly wild-type than the cis-type; that is, *R*(++)/*Cbx Ubx* lacks the very slight Type-IV transformation characteristic of ++/*Cbx Ubx*. The latter result has, moreover, been verified for a number of different *R*'s besides bw^{VDe3}.

It is a general rule that the phenotypic differences involved in the recognition of the trans-vection effects are relatively slight ones. Maximum sensitivity for the detection of such differences requires attention to environmental conditions: constant temperature (25° C) and sufficient food. Combinations of two different rearrangements or single rearrangements more complex than bw^{VDe3}, for example, have been used effectively to increase such differences, as in the case of bx^3 and *pbx*, noted above. Such techniques, as well as the use of sensitizing modifier genes, may help to reveal trans-vection effects in the cases where none have yet been detected. Although isogenic stocks have not been employed, an approach to complete control over the genetic background has been made in most of the observed trans-vection effects by utilizing a variety of stocks of each mutant type (containing different substitutions with respect to closely linked marker genes), and by utilizing different chromosomal rearrangements of independent origin. Such techniques have actually failed to reveal differences traceable to modifier genes; that is, the results are reproducible under a wide variety of genetic backgrounds and with different rearrangements.

To summarize, trans-vection effects have been detected between bx^3 and *Ubx*, bx^3 and *pbx*, bx^3 and *Cbx* (if sensitized by the presence of *su-Cbx*), and *Cbx* and *Ubx*, as well as between bx^{34e} and *Ubx* (as reported in a previous study—Lewis, 1954b). Thus, such effects are not dependent upon the presence of some particular mutant type. The mutants, *Cbx* and *Ubx*, give a unique result in that a phenotypic difference arises only between the *R*(cis)-type and the cis-type; while in all of the other cases, it arises only between the *R*(trans)-type and the trans-type.

DISCUSSION

An attempt will be made below to construct a model of gene-controlled reactions in the case of the bithorax pseudoallelic series which will (1) take account of the principal mutant transformations, (2) explain as simply

as possible the observed cis-vection and trans-vection effects, and (3) give some picture of how the genes control the wild-type segmentation pattern.

In a previous report (Lewis, 1951) it was pointed out that the position effects between bx^3 and *Ubx*, and *Ubx* and *bxd* (cis-vection effects, in the newer terminology), can be simply interpreted on the basis of three successive, gene-controlled reactions occurring at the chromosomal level. The order of control could be postulated to be either: $bx^+ - Ubx^+ - bxd^+$ or $Ubx^+ - bx^+ - bxd^+$. In addition to the finding that this scheme satisfactorily accounted for the phenotype of a great many genotypes, it received independent support from studies of chromosomal rearrangements having one breakage point within the pseudoallelic series itself. Thus, rearrangements which apparently separate bx^+ and Ubx^+ on the one hand from bxd^+, on the other, were found to have no detectable change in the action of the bx^+ and Ubx^+ genes but were found to act like extreme mutant changes in the action of bxd^+. For example, the homozygote for the transposition, bxd^{100} (which has bx^+ and probably Cbx^+ and Ubx^+ transposed to the left arm of the third chromosome) has extreme transformations of the *bxd* type (Type II and Type III), but not of the *bx* type (Type I). On the above scheme the trans-type between bx^3 and *bxd* might also be expected to give the *bxd* types of transformations; while the cis-type would be expected to be wild-type. Although the trans-type as well as the cis-type in the case of bx^3 and *bxd* is wild-type, this was not regarded as necessarily inconsistent with the above scheme since both bx^3 and *bxd* could be regarded as intermediate alleles of their respective loci and, therefore, as mutants which would only partially block their respective gene-controlled reactions. The latter interpretation was especially plausible since (1) *bx*-like mutants of X-ray origin, which are more extreme than bx^3, give strong Type-II and Type-III transformations in the trans-types with *bxd*; and (2) extreme *bxd*-like mutants of X-ray origin, such as bxd^{100}, give a very slight Type-II transformation in the trans-type with bx^3. In neither of these cases could the assumption of a cis-vection be tested, since the complication of chromosomal rearrangements associated with the X-ray induced changes prevented the recovery of cis-types.

The new findings reported here concerning the *pbx* mutant help to clarify the above findings regarding the Type-II transformation, and permit an extension of the above scheme to include the pbx^+ gene. Thus, the Type-II transformations which have just been discussed, as well as those which characterize the trans-types between *pbx* on the one hand, and bx^3, *Ubx*, or *bxd*, on the other, are all readily accounted for if it is assumed that (1) pbx^+ controls a reaction subsequent to that of bxd^+, and (2) a reduction in the concentration of the gene product of pbx^+ leads to the Type-II transformation. This gives the scheme "Scheme 1" (see Figure 1) for the sequence of gene-controlled reactions. Although the order of control of reactions by bxd^+, pbx^+, and either bx^+ or Ubx^+ is the same as the gene order in the chromosome, the order of control with respect to bx^+ and Ubx^+ cannot be deduced from the data and is arbitrarily assumed to be the same as that in the chromosome to simplify the discussion of this scheme.

$$A \xrightarrow{bx^+} B \xrightarrow{Ubx^+} C\text{-}I \xrightarrow{bxd^+} D\text{-}III \xrightarrow{pbx^+} E\text{-}II$$

FIGURE 1

The omission of Cbx^+, whose locus in the chromosome is between those of *bx* and *Ubx*, is intentional, since as discussed below its role in such a scheme is not clear. In the above notation the roman numeral after the symbol of the gene product identifies the type of transformation controlled by that product. More exactly, a reduction in the relative concentration of substance C-I is postulated to lead to a transformation of Type I; of D-III to one of Type III; and of E-II to one of Type II. The substances, C-I and D-III, at least, are assumed to be produced in sufficient concentration to act not only as substrates for the appropriate successive step in the chain, but also as agents which somehow ultimately direct the control of specific physiological processes. The intermediate substances may be thought of as enzymes, as enzyme percursors, or more likely as products of enzymatic activity, that activity in the latter case coming either directly from the gene or from an enzyme closely held to the gene. The essential requirement for the interpretation of the observed cis-vection and trans-vection effects is that these substances be produced at, or very close to, the site of the genes in the chromosome, rather than at other points in the cell.

Specifically, the Type-II transformations discussed above are accountable for on Scheme 1 in the following way. In the case of the trans-type, $bx^3+/+bxd^{100}$, the bx^3 mutant is assumed to reduce relatively slightly the concentration of B, which then leads indirectly to a relatively slight reduction in E-II; in the other chromosome, the separation of bx^+ and pbx^+ is expected to result in a relatively extreme reduction in the concentration of E-II; the observed very slight Type-II transformation is thus accounted for. In the case of $bx^3+/+pbx$, bx^3 is assumed to act as in the preceding case, while a relatively extreme reduction in E-II in the other chromosome is postulated to result from a blocking of the final reaction by the *pbx* mutant; the observed result, that a very slight Type-II transformation arises in this case only if there is at the same time structural heterozygosity, will be discussed below. In the case of $Ubx+/+pbx$, the *Ubx* mutant is assumed to cause a relatively extreme reduction in C-I and in turn of E-II, while the *pbx* would act as in the previous case; the observed extreme Type-II transformation is thus accounted for. Finally, in the case of $bxd+/+pbx$, the *bxd* mutant is assumed to cause a moderate reduction of D-III and therefore in turn of E-II; while the *pbx* mutant would act as before; the observed moderate Type-II transformation is thus accounted for. In the case of the three respective cis-types, $bx^3pbx/++$, $Ubxpbx/++$, and $bxdpbx/++$, the chromosome carrying the wild-type alleles of the pseudoallelic genes is assumed to produce its normal quota of substance E-II and this quota is assumed to be sufficient to prevent the Type-II transformation, even though the other chromosome might fail to produce any E-II; the observed failure of the cis-types to show a Type-II transformation is thus accounted for. The remain-

ing cis-vection effect comparisons involving the four loci shown in Scheme 1 are those involving the following pairs: bx^3 and *Ubx*, bx^3 and *bxd*, and *Ubx* and *bxd*. As already noted these cases have been discussed elsewhere (Lewis, *loc. cit.*) in detail in terms of a model including the first three steps of Scheme 1. The only change that Scheme 1 introduces is to interpret Type-II transformations as being controlled not by the product of the bxd^+ gene, but by that of pbx^+. In the case of $bx^3+/+bxd$, it remains necessary to postulate a threshold effect to explain its wild-type phenotype; in other words, the reduction in amount of substances D-III and E-II which is expected in this genotype on Scheme-1 is postulated to be below the threshold required to give a detectable mutant phenotype.

The application of the sequential reaction type of interpretation to trans-vection effects has been discussed elsewhere (Lewis, 1954b) and will only be briefly considered here. In the case of $bx^3+/+Ubx$, for example, the Ubx^+ gene is assumed on Scheme-1 to receive some of its substrate B from the action of the bx^3 gene and some from diffusion of B from its site of production in the homologous chromosome; while in the presence of structural heterozygosity, the Ubx^+ gene is assumed to be deprived (relatively speaking) of this latter source of B as the result of reduced somatic pairing of the homologous chromosomes at that site. A similar argument would apply to the remaining trans-vection effect observed in Groups 1 and 2 of table 2; namely, that involving bx^3 and *pbx*; here, however, the substance(s) involved might be any of the intermediates: B, C-II and/or D-III.

It is important to consider how, conversely, the production of substances C-I, D-III and E-II would be expected to control the development of the wild-type segmentation pattern. This should provide a test of the utility of Scheme-1 and an aid to the understanding of the mutant phenotypes. Thus, the production of substances C-I and E-II is postulated to cause the wild-type metathoracic segment, to advance from a primitive level of developmental determination, which will be designated as a mesothoracic-like level, "L-ms," to a metathoracic-like level, or "L-mt." On evolutionary grounds, L-ms is more primitive than L-mt, since the Diptera almost certainly evolved from four-winged ancestors. A reduction in concentration of C-I or E-II would be expected on this basis to cause AMT or PMT, respectively, to tend to remain at the level, L-ms, which agrees with the respective definitions of the Type-I and Type-III transformations.

The production of substance D-III is postulated to cause the first abdominal segment of the wild-type organism to change from a primitive level, which again will be designated L-ms, to a first-abdominal level, or "L-ab_1." On evolutionary grounds, L-ms is probably more primitive than L-ab, since the ancestors of the insects certainly had legs on the abdominal segments, and the immature stages of many insects bear ventral abdominal appendages. In an earlier discussion of these levels of developmental determination (Lewis, 1951) it was assumed that this product of the bxd^+ gene (D-III) causes the first abdominal segment to change in a way that would be the exact reverse of the Type-III transformation ($AB_1 \rightarrow MT$); that is, causes

change: L-mt → L-ab$_1$. This earlier assumption is unsatisfactory on evolutionary grounds and does not give a clear picture of the way in which development of this segment would take place in certain genotypes; it will therefore be discarded in favor of the above assumption that the production of D-III leads to the change: L-ms → L-ab$_1$. In other words, the first abdominal segment is postulated to develop according to two alternate pathways, rather than according to two successive pathways (namely, L-ms → L-mt → L-ab$_1$), as formerly assumed. The alternate pathways and their specific controlling substances will be designated Scheme-2, and may be represented as follows:

$$\text{L-mt} \xleftarrow{\text{C-I + E-II}} \text{L-ms} \xrightarrow{\text{D-III}} \text{L-ab}_1$$

This scheme gives for the first time an adequate explanation of the development of AB$_1$ in the principal mutant types as well as in the wild-type organism. The following examples illustrate the way in which Scheme-2 is assumed to apply in such cases (see table 1 for the observed effects). Thus, the *bx*^{3} homozygote possesses a sufficient quantity of substance D-III to direct AB$_1$ along the pathway to L-ab$_1$. The *bxd* homozygote lacks a sufficient quantity of D-III to direct the segment along this latter pathway, and lacks sufficient E-II to direct the posterior portion towards PMT, so that this portion should remain at level, L-ms; however, it has sufficient C-I to direct the anterior portion towards the level, L-mt. Finally, homozygotes for double mutants between *bx* and *bxd* mutants, or for *Ubx*, lack sufficient quantities of all three substances, so that AB$_1$ should remain at the level, L-ms.

The remaining segment of the wild-type organism to be considered in connection with Schemes 1 and 2 is the mesothorax. It is presumed that Scheme 1 does not effectively come into play during the development of this segment, perhaps because of an anterior-posterior gradient in the distribution of the initial substrate A. Thus, lack of sufficient quantities of substances C-I, D-III and E-II would leave MS at its primitive level of development, L-ms.

Scheme-1 has been found to account adequately for all of the genotypes constructed to date involving the *bx*^{3}, *Ubx*, *bxd* and *pbx* mutants, except for one case: *bx*^{3}++/+*Ubx bxd*. (Some 56 such genotypes among a possible total of 136 have been constructed, the remaining ones involving chiefly triple and quadruple mutant combinations, which have not yet been synthesized.) This exceptional case, omitted above since it is a triple mutant heterozygote, has a more extreme Type-I, and a less extreme Type-II transformation than does the genotype, *bx*^{3}++/+*Ubx*+. On Scheme-1 it would be expected that the former genotype would, if it did so at all, differ from the latter by having a less extreme Type-I transformation, as a result of the possibility of an accumulation of substance C-I, and a more extreme Type-II transformation, as a result of two blocks along the pathway to E-II in the

+ *Ubx bxd* chromosome, compared to only one in the + *Ubx* + chromosome. For in other cases, just such predictions are verified; for example, bx^3 + *bxd*/bx^{34} ++ has a less extreme Type-I transformation than bx^3 ++/bx^{34e} ++ (where bx^{34e} is an intermediate *bx* allele); and *Ubx bxd*/+ *bxd* has a more extreme Type-II transformation than does *Ubx* +/+ *bxd*.

The above difficulty in interpreting one exceptional genotype on Scheme-1 has not been resolved and indicates that at least one more variable is needed, if it is to remain a valid working hypothesis. There is such a variable in the case of substance B, since it has not been assigned a specific function (other than as substrate for the Ubx^+ gene). There is also the question of the role of Cbx^+ in Scheme 1, since this gene and its product constitute an additional variable, yet to be considered. But just what role Cbx^+ may play is not clear. Many of the interactions of the *Cbx* mutant with other mutants of the series indicate that Cbx^+ may control an additional step near the beginning of the reaction sequence; while its dominant effect may be attributed to an accumulation of some product such as B or C-I which would begin to cause MS to develop towards MT. However, since the *Cbx* transformation (Type IV) is the inverse of those of Type I and Type II, the possibility of complicated interactions at a physiological level between the numerous hypothetical gene products involved in such transformations becomes an acute one.

The unique finding of a trans-vection effect by comparing cis- and *R*(cis)-types involving *Cbx* and *Ubx* can be formally reconciled with the other trans-vection effects, even though the role of *Cbx* in Scheme-1 is not clear. Thus, it may be that in *Cbx Ubx*/++ a higher concentration of substance B reaches the *Ubx* gene than does so in *R*(++)/*Cbx Ubx*, where the homologous chromosomes are relatively farther apart; this would mean more of substance C-I, or a more nearly normal Type-I transformation, in the cis-type than in the *R*(cis)-type; in turn this would lead, paradoxically, to the observed result that the cis-type has a more extreme Type-IV transformation than the *R*(cis)-type, since the available evidence suggests that the more extreme the Type-I change the less extreme the Type-IV change.

CONCLUSIONS

It is concluded that there is an ordered complexity to the variety of cis-vection and trans-vection effects, as well as individual mutant effects, in the case of the bithorax pseudoallelic series. The "functional" interpretation has to explain such results in terms of a concept of a single functional unit of probably considerable complexity—analogous to the old concept of the gene as deduced from the behavior of complex "multiple allelic" series. The "genetic" interpretation explains such results in terms of a working hypothesis in which each of the component genes of the series acts as a functional unit in the modern sense—namely, as an agent controlling a single, specific reaction.

SUMMARY

Contrasting interpretations of position pseudoallelism are discussed, and the types of position effect which characterize this phenomenon are illustrated, with special reference to the case of the bithorax series of five pseudoallelic loci in *Drosophila melanogaster*. Most of the results in this latter case can be simply interpreted on the basis of a chain of gene-controlled reactions in which each intermediate substance is postulated to act in a dual capacity: as substrate for the succeeding reaction and as a determiner of a specific physiological process. The model also gives a consistent picture of the way in which different levels of developmental determination may be controlled by the different genes of the series.

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